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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/525,019	GIESING ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 May 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *26-30,32,34,36-60 and 62-70* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *26-30,32,34,36-60 and 62-70* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *18 February 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This action is in response to the amendment, filed 5/12/2010, in which claims 31, 33, 35 and 61 were canceled, claims 26, 45, 47, 48, 50, 66 and 68 were amended, and claims 69 and 70 were newly added. Claims 26-30, 32, 34, 36-60, 62-70 are pending.

Applicant's arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections and objections not reiterated in this action have been withdrawn. **This action is FINAL.**

Election/Restrictions

Applicant elected Group I with traverse in the reply filed on 1/28/2008.

Claims 26-30, 32, 34, 36-60, 62-70 are under consideration.

Response to Arguments - Claim Objections

The objection of claim 31 is moot in view of Applicant's cancellation of the claim in the reply filed 5/12/2010.

The objections of claims 66-68 have been withdrawn in view of Applicant's amendment to the claims in the reply filed 5/12/2010.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 26-30, 32, 34, 36-60 and 62-70 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for (a) obtaining a blood sample from a human subject, collecting mononuclear cells from the blood sample, removing a fraction of the mononuclear cells to obtain test fraction A', passing the remaining mononuclear cells through a screen with a 20 μm mesh, and collecting cells from the mesh to obtain test fraction C;

(b) obtaining blood samples from a healthy human subjects not suffering from cancer, collecting mononuclear cells from the blood samples, removing a fraction of the mononuclear cells to obtain reference fraction A', passing the remaining mononuclear cells through a screen with a 20 μm mesh, and collecting cells from the mesh to obtain reference fraction C;

(c) isolating CD45-positive lymphocytes from reference fraction A' to obtain reference fraction A,

(d) isolating mRNA from test fraction A', test fraction C, reference fraction A, and reference fraction C;

(e) measuring the expression level of manganese superoxide dismutase (MNSOD), thioredoxin reductase (TXNRD1), and glutathione peroxidase (GPX1) in each of the mRNA samples, wherein said measuring is by reverse transcription and PCR using primers consisting of SEQ ID NOs: 1 and 2 for MNSOD, SEQ ID NOs: 4 and 5 for TXNRD1, and SEQ ID NOs: 7 and 8 for GPX1;

(f) determining the average and standard deviation for the expression ratio of MNSOD, TXNRD1, and GPX1 from reference fraction C to reference fraction A for the healthy control samples, and determining a limit for expression which is the average plus one standard deviation;

(i) determining the expression ratio of MNSOD, TXNRD1, and GPX1 from test fraction C to test fraction A' of the test sample; and

(j) comparing the expression ratio for each of MNSOD, TXNRD1, and GPX1 for the test sample to the determined limit for each gene;

wherein an expression ratio higher than the limit for at least one of MNSOD, TXNRD1 or GPX1 indicates that disseminated cancer cells are present in the test blood sample,

does not reasonably provide enablement for the use of bone marrow or any body fluid other than blood, practice of the method without the use of cancer cell enrichment with a mesh containing 20 μm pores, a further-cell containing fraction as a reference sample, the use of an undefined average ratio from subjects not having cancer, determining the expression of any manganese superoxide dismutase gene, any thioredoxin reductase gene, or any glutathione peroxidase gene, or measuring protein levels to indicate the presence of disseminated cancer cells in the body fluid. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims. This rejection was made in the Office action mailed 11/12/2009 and has been rewritten to address the amendments to the claims in the reply filed 5/12/2010.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the invention: Claims 26-30, 32, 34, 36-47, 63 and 65 are drawn to a method for investigating a body fluid from a human subject having or suspected of having cancer for disseminated cancer cells. Claim 26 is drawn to the following method steps: (1) obtaining a cell-containing fraction from the body fluid with enrichment of cancer cells and determining in the cell-containing fraction from the expression of at least 2 genes which are selected from the group consisting of (i) a human manganese superoxide dismutase gene; (ii) a human thioredoxin reductase 1 gene; and (iii) a human glutathione peroxidase 1 gene, wherein the body fluid is blood or bone marrow; (2) providing a further cell-containing fraction of the body fluid from the same individual and determining the expression of the genes in the further cell-containing fraction; and (3) comparing the expression for each of said at least 2 genes in the cell-containing fraction with its expression in the further cell-containing fraction, wherein an elevated expression of at least one of said at least 2 genes determined in the cell-containing fraction, as compared to the further cell-containing fraction indicates the presence of disseminated cancer cells in the body fluid. The nature of the invention is complex in that increased expression of at least one of the claimed genes must be indicative of disseminated cancer cells in blood or bone marrow when any cell-containing fraction is compared to another cell-containing fraction from the same individual.

The dependent claims further limit the genes that are measured. Dependent claim 27 requires the expression of a manganese superoxide dismutase gene, a thioredoxin reductase gene and a glutathione peroxidase 1 gene to be determined. Claim 30 requires the expression of a manganese superoxide dismutase gene and at least one further gene selected from a thioredoxin reductase 1 genes and a glutathione peroxidase 1 genes to be determined. Claim 36 requires the

manganese superoxide dismutase gene to encode a protein that has the amino acid sequence of SEQ ID NO: 13 or an allelic variant thereof. Claim 37 requires the manganese superoxide dismutase gene to encode an mRNA which is capable of being amplified using the primer sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 2. Claim 38 requires the thioredoxin reductase 1 gene to encode a protein having the amino acid sequence of SEQ ID NO: 15 or an allelic variant thereof. Claim 39 requires the thioredoxin reductase 1 gene to encode an mRNA which is capable of being amplified using the primer sequences set forth in SEQ ID NO: 4 and SEQ ID NO: 5. Claim 40 requires the human glutathione peroxidase 1 gene to encode a protein having the amino acid sequence of SEQ ID NO: 17 or an allelic variant thereof. Claim 41 requires the human glutathione peroxidase 1 gene to encode an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 7 and SEQ ID NO: 8. Claim 42 specifically requires determining the expression levels by determining mRNA expressed by the genes.

The dependent claims further limit the step of obtaining a cell-containing fraction. Dependent claim 28 limits the body fluid to blood. Claim 29 limits the step of obtaining the cell-containing fraction to passing the body fluid or a cell-containing fraction thereof through a screen with a mesh or pore width of about 10 to 200 μm and obtaining the cell fraction retained on the screen. Claim 46 limits the method of claim 29 to the method where the screen has a mesh or pore width of about 20 μm. Claims 43 and 44 require the step of isolating cancer cells. The nature of claims is complex in that they require the isolation of cancer cells from a subject only suspected of having cancer. Claim 45 limits the cell-containing fraction to one that is derived from blood and comprises mononuclear cells.

Claims 48 and 49 are directed to a method for investigating a body fluid from a human subject having or being suspected of having cancer for disseminated cancer cells. The method of claim 48 comprises the following steps: (1) obtaining a cell-containing fraction from the body fluid by passing the body fluid or a cell-containing fraction thereof through a screen with a mesh or pore width of about 20 μm and obtaining the cell fraction retained on the screen, wherein the body fluid is blood or bone marrow; (2) determining in the cell-containing fraction the expression of (i) a human manganese superoxide dismutase gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 1 and SEQ ID NO: 2, wherein the manganese superoxide dismutase gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 13 or an allelic variant thereof; (ii) a human thioredoxin reductase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 4 and SEQ ID NO: 5, wherein the thioredoxin reductase 1 gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 15 or an allelic variant thereof; and (iii) a human glutathione peroxidase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 7 and SEQ ID NO: 8, wherein the human glutathione peroxidase 1 gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 17 or an allelic variant thereof; (3) providing a further cell-containing fraction of the body fluid from the same individual and determining the expression of the genes in the further cell-containing fraction of the body fluid; and (4) comparing the expression for each of said at least 2 genes in the cell-containing fraction with its expression in the further cell-containing fraction, wherein an elevated expression of at least one of said at least 2 genes in the cell-containing fraction as compared to its expression in the further

cell-containing fraction indicates the presence of disseminated cancer cells in the body fluid if the ratio of its expression in the cell-containing fraction to its expressing in the further cell-containing fraction is significantly higher than the average ratio of its expression than the corresponding average ratio of its expression in subjects not having cancer. Claim 49 depends from claim 48 and requires the following: (a) the body fluid is blood; (b) the cell-containing comprises mononuclear cells; (c) the further cell-containing fraction comprises mononuclear cells; and (d) determining the expression comprises determining mRNA expressed by the gene. The nature of the invention is complex in that increased expression of at least one of the claimed genes must be indicative of disseminated cancer cells in blood or bone marrow when any cell-containing fraction is compared to another cell-containing fraction from the same individual or where blood-derived MNC fractions are compared. The ratios are then compared to any "corresponding average ratio of its expression in subjects not having cancer" where the "corresponding average ratio" is not necessarily obtained from cells enriched from blood over a 20 μm mesh and CD45-positive cells obtained from mononuclear cells of blood.

Claims 50-60 and 64 are drawn to a method for investigating a body fluid from a human subject having or being suspected of having cancer for disseminated cancer cells. Claim 50 sets forth the following method steps: (1) obtaining a cell-containing fraction from the body fluid and determining in the cell-containing fraction the expression of at least 2 genes which are selected from the group consisting of (i) a human manganese superoxide dismutase gene; (ii) a human thioredoxin reductase 1 gene; and (iii) a human glutathione peroxidase 1 gene, where the body fluid is blood or bone marrow; and (2) comparing the expression for each of said at least 2 genes in the cell-containing fraction with its average expression in subjects not having cancer,

wherein significantly higher expression of at least one of said at least 2 gene sin the cell-containing fraction as compared to its average expression in subjects not having cancer indicates the presence of disseminated cancer cells in the body fluid. The nature of the invention is complex in that it does not require any enrichment of potential cancer cells but requires significantly higher expression to be detected where the cells with higher expression may only be present at a ratio of 1:1000. 1:10000 or 1:100000 relative to the mononuclear cells present (specification, page 6, lines 15-29).

The dependent claims further limit the genes that are measured. Claim 51 requires the expression of a manganese superoxide dismutase gene and at least one further gene selected from a thioredoxin reductase 1 genes and a glutathione peroxidase 1 genes to be determined. Claim 52 requires the expression of a manganese superoxide dismutase gene, a thioredoxin reductase gene and a glutathione peroxidase 1 gene to be determined. Claim 55 requires the manganese superoxide dismutase gene to encode a protein that has the amino acid sequence of SEQ ID NO: 13 or an allelic variant thereof. Claim 56 requires the manganese superoxide dismutase gene to encode an mRNA which is capable of being amplified using the primer sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 2. Claim 57 requires the thioredoxin reductase 1 gene to encode a protein having the amino acid sequence of SEQ ID NO: 15 or an allelic variant thereof. Claim 58 requires the thioredoxin reductase 1 gene to encode an mRNA which is capable of being amplified using the primer sequences set forth in SEQ ID NO: 4 and SEQ ID NO: 5. Claim 59 requires the human glutathione peroxidase 1 gene to encode a protein having the amino acid sequence of SEQ ID NO: 17 or an allelic variant thereof. Claim 60 requires the human glutathione peroxidase 1 gene to encode an mRNA which is capable of being amplified using the

primer sequences as set forth in SEQ ID NO: 7 and SEQ ID NO: 8. Claim 54 specifically requires determining the expression levels by determining mRNA expressed by the genes.

Claims 66 and 67 are drawn to a method for investigating a body fluid for disseminated cancer cells in a human subject having or being suspected of having cancer. Claim 66 sets forth the following method steps: (1) obtaining a cell-containing fraction from the body fluid and determining in the cell-containing fraction the expression of (i) a human manganese superoxide dismutase gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 1 and SEQ ID NO: 2, wherein the manganese superoxide dismutase gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 13 or an allelic variant thereof; (ii) a human thioredoxin reductase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 4 and SEQ ID NO: 5, wherein the thioredoxin reductase 1 gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 15 or an allelic variant thereof; and (iii) a human glutathione peroxidase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 7 and SEQ ID NO: 8, wherein the human glutathione peroxidase 1 gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 17 or an allelic variant thereof, wherein the body fluid is blood or bone marrow; and (2) comparing the expression for each of said at least 2 genes in the cell-containing fraction with its average expression in subjects not having cancer, wherein significantly higher expression of at least one of said at least 2 genes in the cell-containing fraction as compared to its average expression in subjects not having cancer indicates the presence of disseminated cancer cells in the body fluid. Claim 67 depends from claim 66 and requires (i) the body fluid is blood;

(ii) the cell-containing fraction comprises mononuclear cells; and (iii) determining gene expression comprises determining mRNA expressed by the gene. The nature of the invention is complex in that it does not require any enrichment of potential cancer cells but requires significantly higher expression to be detected where the cells with higher expression may only be present at a ratio of 1:1000. 1:10000 or 1:100000 relative to the mononuclear cells present (specification, page 6, lines 15-29).

Claim 68 is drawn to a method for investigating a blood or bone marrow sample for disseminated cancer cells in a human subject having or suspected of having cancer. The claim sets forth the following method steps: (a) obtaining a blood or bone marrow sample from the human to obtain a test fraction; (b) obtaining a blood or bone marrow sample from a healthy human subject not suffering from cancer to obtain a reference fraction; (c) isolating mRNA from the test fraction and reference fraction to obtain an mRNA test sample and an mRNA reference sample, respectively; (d) measuring the expression level of human manganese superoxide dismutase (MNSOD), human thioredoxin reductase 1 (TXNRD1), and human glutathione peroxidase 1 (GPX1) in the mRNA sample and the mRNA reference sample, wherein the measuring is by reverse transcription and PCR with primers selected from the nucleotides of SEQ ID NOs: 1 and 2 for MNSOD; SEQ ID NOs: 3 and 4 for TXNRD1; and SEQ ID NOs: 7 and 8 for GPX1; and (e) comparing the expression of MNSOD, TXNRD1, and GPX1 in the mRNA test sample to the mRNA reference sample, and wherein a higher expression of MNSOD, TXNRD1 and GPX1 in the mRNA test sample as compared to the mRNA reference sample indicates the presence of disseminated cancer cells in the blood or bone marrow samples from the human subject having or suspected of having cancer. The nature of the invention is complex

in that it does not require any enrichment of potential cancer cells but requires significantly higher expression to be detected where the cells with higher expression may only be present at a ratio of 1:1000. 1:10000 or 1:100000 relative to the mononuclear cells present (specification, page 6, lines 15-29).

Claim 69 is drawn to a method for investigating a body fluid from a human subject having or being suspected of having cancer for disseminated cancer cells. The claim sets forth the following method steps: (a) obtaining a cell-containing fraction from the body fluid with enrichment of cancer cells; (b) determining in the cell-containing fraction the expression of (i) a human manganese superoxide dismutase gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 1 and SEQ ID NO: 2, wherein the manganese superoxide dismutase gene encodes a protein having an amino acid sequence as set forth in SEQ 1D NO: 13 or an allelic variant thereof; (ii) a human thioredoxin reductase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ 1D NO: 4 and SEQ ID NO: 5, wherein the thioredoxin reductase 1 gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 15 or an allelic variant thereof; and (iii) a human glutathione peroxidase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO:7 and SEQ ID NO: 8, wherein the human glutathione peroxidase 1 gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 17 or an allelic variant thereof; (c) providing a further cell-containing fraction of the body fluid from the same individual and determining the expression of the genes in the further cell-containing fraction; and (d) comparing the expression for each of said at least 2 genes in the cell-containing fraction with its expression in the further

cell-containing fraction, wherein the body fluid is blood and the cell-containing fraction and the further cell-containing fraction comprise mononuclear cells; wherein determining the expression of the at least 2 genes comprises determining mRNA expressed by the gene; and wherein an elevated expression of at least one of said at least 2 genes in the cell-containing fraction as compared to its expression in the further cell-containing fraction indicates the presence of disseminated cancer cells in the body fluid if the ratio of its expression from the cell-containing fraction to the further cell-containing fraction is significantly higher than the average ratio of its expression in subjects not having cancer.

Claim 70 is drawn to a method for investigating a body fluid from a human subject having or being suspected of having cancer for disseminated cancer cells. The claim sets forth the following method steps: (a) obtaining a cell-containing fraction from the body fluid with enrichment of cancer cells; (b) determining in the cell-containing fraction the expression of (i) a human manganese superoxide dismutase gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 1 and SEQ ID NO: 2, wherein the manganese superoxide dismutase gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO:13 or an allelic variant thereof; ii) a human thioredoxin reductase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO:4 and SEQ ID NO:5, wherein the thioredoxin reductase 1 gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO:15 or an allelic variant thereof; and (iii) a human glutathione peroxidase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 7 and SEQ ID NO: 8, wherein the human glutathione peroxidase 1 gene encodes a protein having an amino

acid sequence as set forth in SEQ ID NO: 17 or an allelic variant thereof; and comparing the expression for each of said at least 2 genes in the cell- containing fraction with its average expression in subjects not having cancer, wherein the body fluid is blood and the cell-containing fraction comprises mononuclear cells; wherein determining the expression of the at least 2 genes comprises determining mRNA expressed by the gene; and wherein a significantly higher expression of at least one of said at least 2 genes in the cell-containing fraction as compared to its average expression in subjects not having cancer indicates the presence of disseminated cancer cells in the body fluid.

The nature of the invention is complex in that carrying out the recited method steps must enable the intended uses of the method, including identifying disseminated cancer cells in a body fluid, providing a diagnosis of a tumor, and estimating the risk to develop a metastasis or recurrence.

Breadth of the claims: The claims are broad in that the specification defines the term "cancer cell" to mean a cell which exhibits one or more modifications associated with cancer, i.e., dysplasia in the general sense. The term is defined to specifically include precursors of cancer and tumor cells with cancerous or tumorous modifications (e.g., page 4, lines 6-19).

The claims are very broad in that they encompass determining the expression of at least two genes selected from manganese superoxide dismutase genes, thioredoxin reductase 1 genes, and glutathione peroxidase 1 genes. The specification defines the term "manganese superoxide dismutase (MNSOD)" to mean enzymes which catalyze the decomposition of superoxide free radicals to form hydrogen peroxide, and in particular the enzymes which constitute enzyme class 1.15.1.1 (paragraph bridging pages 14-15). The enzymes of this class are not limited to

manganese-containing superoxide dismutase enzymes (See the entry for 1.15.1.1 from the Enzyme nomenclature databases, accessed from http://us.expasy.org/enzyme, cited in a prior action). Enzymes of the class 1.15.11 include all superoxide dismutase enzymes, including iron or manganese or copper and zinc superoxide dismutase. Thus, the claims read on determining the expression level of any superoxide dismutase enzyme from any species of organism from which a body fluid may be obtained. The claims read on determining the expression level of any thioredoxin reductase 1 isoform from any species of organism from which blood or bone marrow may be obtained. The claims read on determining the expression level of any glutathione peroxidase isoform from any species of organism from which blood or bone marrow may be obtained. Accordingly, the claims broadly encompass obtaining blood or bone marrow from any species of organism, and determining the expression of at least two of the broadly defined classes of genes selected from the genus of manganese superoxide dismutase genes, the genus of thioredoxin reductase 1 genes, and the genus of glutathione peroxidase 1 genes. Claims 66 and 67 do not require the use of the primers in the claimed method. The gene must only be capable of being amplified by the primer sets. As shown in Exhibits I and II (mailed 1/5/2009), the primers of SEQ ID NOS: 7 and 8 are capable of amplifying genes from a number of different species.

The claims are broad in that the comparable biological sample may be from any body fluid or solid tissue of any subject or "corresponding" cell-containing fractions, where the nature of the correspondence isn't limited. Accordingly, the claims encompass a large number of different comparisons between the tested cell-containing fraction and a further cell-containing fraction or a comparable biological sample.

The complex nature of the subject matter of this invention is greatly exacerbated by the breadth of the claims.

Guidance of the specification and existence of working examples: The specification envisions using a method for investigating body fluids for cancer cells to permit reliable tumor diagnosis and prognosis (e.g., page 1, lines 5-13).

The specification teaches that the prior art shows that some solid tumors and metastases thereof found in solid tissue have increased expression of MNSOD, including colorectal tumors and hepatic metastases thereof, lung tumors, breast cancer cells, stomach tumors, and gliobalastoma (e.g., page 2, lines 5-22). However, the specification also notes that benign hyperplasias of the breast were often found to be strongly positive for MNSOD expression as compared to neoplastic epithelial cells from invasive carcinomas of the breast (e.g., page 2, lines 22-27). Thus, the specification acknowledges that MNSOD levels are not always higher in dysplastic cells as compared to any cell type. The specification teaches that reduced GPX1 expression was observed in imexon-resistant RPM/8226/I myeloma cells (e.g., page 3, lines 10-12). Thus, gene expression may vary depending upon the sensitivity or resistance of the cancer cell to a cancer therapeutic. Further, the specification teaches that disseminated cancer cells are a tumor entity independent of the primary tumor and therefore are fundamentally different from cells of the primary tumor on the basis of a different genotype and phenotype (e.g., page 3, lines 14-22).

At pages 12-27, the specification provides general guidance directed to measuring expression levels of MNSOD, TXNRD and GPX expression by measuring nucleic acid or protein expression.

At pages 28-30, the specification provides guidance with regard to evaluating the obtained expression levels. The specification teaches that it is particularly important to determine whether expression in the cells of the investigated sample is comparatively elevated (e.g., page 28, lines 1-10). The specification teaches that the comparison usually is with cells in which no cancer-associated modification is to be expected (non-cancer cells, normal cells) (e.g., page 28, lines 10-14). The specification suggests that if cancer cells in body fluids are being tested, then the comparison will be those normally occurring in this body fluid. For the case of blood, the normal cells are white blood cells which can be obtained for example by density gradient centrifugation (e.g., the buffy coat or the MNC fraction) or can be separated by more specific isolation methods (e.g., CD45-positive lymphocytes) (e.g., page 28, lines 14-22). The specification asserts that these samples can also be used as a comparison for body fluids other than blood (e.g., page 28, lines 22-25). At page 29, lines 33-39, the specification states, "The test principle according to the invention is therefore based on determining whether enrichment of cancer cells is associated with a measurable increase in MNSOD, TXNRD and GPX expression. The ratio of the expression measured in the test cell mixture to the expression measured in the comparison cell mixture is decisive." The specification goes on to state, "It will usually be expedient for validation of a particular test system to fix a particular quotient (limit) above which overexpression is present by definition." (See page 30, lines 1-5). Thus, the step of comparing appears to be critical to the claimed invention. Furthermore, the specification notes that the limit may depend on the cell mixtures used and, in particular, on the obtaining thereof (e.g., page 30, lines 7-8).

With respect to early diagnosis, the specification envisions using sputum/saliva for the early diagnosis of lung tumors; urine for the early diagnosis of prostate and bladder tumors; stool for the early diagnosis of colonic and pancreatic tumors; and blood/bone marrow/lymph for the early diagnosis of all disseminating tumors.

With respect to the prognosis and risk of recurrence, the specification envisions using the method of the invention to classify tumor and estimate risk (e.g., paragraph bridging pages 31-32).

The working examples of the specification are directed to one embodiment that falls within the scope of the instant claims. The examples teach the collection of blood from 9 healthy donors and 47 tumor patients. Breast carcinoma cell line BT474 was used as a reference for MNSOD, TXNRD1, and GPX1 expression. To obtain cancer cell fraction C and comparative fractions A' and B', 10 ml of heparinized blood was centrifuges, and the supernatant plasma was removed. The pelleted cells were resuspended in 12 ml of PBS and subjected to density gradient centrifugation. The mononuclear cell fraction was collected, washed and resuspended in 10 ml of PBS. 1 ml of this cell mixture was removed as a possible reference (comparative fraction A'). The remaining 9 ml of cell mixture was passed via a column through a screen woven from polyester filaments with a 20 μm mesh width, and the flow-through from the screen was collected as a possible reference (cell fraction B'). The column was washed five times with 10 ml of PBS, and the cells trapped on the screen were collected in Trizol® solution (cancer cell fraction C). Comparative fractions A' and B' were further processed by isolating CD45-positive lymphocytes to obtain comparative fractions A and B. Gene expression was analyzed by TaqMan® analysis of mRNA expression using the following primers and probes: SEQ ID NO: 1

(sense primer for MNSOD), SEQ ID NO: 2 (antisense primer for MNSOD), SEQ ID NO: 3 (probe for MNSOD), SEQ ID NO: 4 (sense primer for TXNRD1), SEQ ID NO: 5 (antisense primer for TXNRD1), SEQ ID NO: 6 (probe for TXNRD1), SEQ ID NO: 7 (sense primer for GPX1), SEQ ID NO: 8 (antisense primer for GPX1), SEQ ID NO: 9 (probe for GPX1), SEQ ID NO: 10 (sense probe for GAPDH), SEQ ID NO: 11 (antisense probe for GAPDH), and SEQ ID NO: 12 (probe for GAPDH). The specification refers to the following accession numbers for MNSOD, TXNRD1, and GPX1: M36693, X91247, and M21304, respectively. GAPDH expression was measured for fractions A or A' and C, and the ratio of the expression of each gene is expressed as a quotient. The specification teaches that overexpression of the relevant mRNA is present if the ratio of the fraction C quotient to the fraction A quotient is more than a limit which is to be experimentally defined. Further, the specification teaches that cell equivalents are based on a cell standard (e.g., cell line BT474), where cDNA from the cell standard is included in the quantitative analysis in the form of serial dilutions and serves as a reference system (e.g., page 44, line 31 to page 45, line 1). The specification teaches the amounts of MNSOD, TXNRD1 and GPX1 mRNA determined in fraction C as compared to fraction A for healthy donors (e.g., Table 1). The specification teaches that for subsequent assessment of the levels of expression measured in tumor patients, levels were regarded as positive if they exceeded the average level in healthy donors (ratio of level in fraction C as compared to fraction A) plus one standard deviation, as indicated as the limit in Table 1 (e.g., page 45, lines 13-26). MNSOD, TXNRD1 and GPX1 was measured in fractions C and A' obtained from the blood of patients diagnosed with a solid tumor (e.g., page 46, lines 1-8). Comparing the expression ratios from fractions C and A' to the limits disclosed in Table 1, it was

determined that 78/90 (87%) patients were positive for increased MNSOD expression, 60/90 (67%) of patients were positive for increased TXNRD1 expression, and 53/86 (62%) of patients were positive for GPX1. At least one gene was positive in 93% of patients. Thus, detecting all three genes has a sensitivity of 93%, while the sensitivity of the individual detections is 87, 67 and 62%, respectively (e.g., page 47, lines 25-30). Comparison between the healthy donors and some of the tumor patients is shown at pages 50-51. The specification teaches the use of this specific method to detect disseminated cancer cells in patients with solid tumors.

The specification does not teach the stage or grade of the cancers at the time blood was drawn. There is no indication that the cancer cells detected by increased expression of MNSOD, TXNRD1 or GPX1 are not a result of advanced metastatic cancer. The specification does not teach the sensitivity of the assay for early, non-metastatic cancer.

With respect to estimating the risk to develop metastasis or recurrence, the specification teaches the comparison between tumor patients with out recurrence and those with recurrence in relation to MNSOD, TXNRD1, and GPX1 expression as discussed above (e.g., pages 52-53). While some statistical differences were observed, the percentages disclosed in Table 7a for carcinoma of the breast and Table 7b for tumor patients, indicates that may not be able to use the expression levels of MNSOD, TXNRD1 and/or GPX1 to reliably classify a single test individual as at risk or not at risk of recurrence. The statistics presented are for groups of patients. All patient groups tested have levels higher than that of the normal controls (see tables). Thus, the indication of a tumor, a recurrence or progression is not based upon the presence or absence of disseminated cancer cells in the blood. Rather, the specification seeks to further classify those individuals with disseminated cancer cells.

The specification discloses probes that could be used for microarray analysis of MNSOD, GPX2, GPX3, and TXNRD1 (e.g., page 55). The specification asserts that overexpression of MNSOD and GPX2 is clearly evident upon hybridization of mRNA total amplification from a tumor cell fraction C as compared to cell fraction A' (e.g., page 57 and Figure 1).

The specification does not teach the expression of MNSOD, TXNRD or GPX in body fluids such as bone marrow, lymph, sputum, lavages, puncture fluids, ascites, mucosal smears, exudates, urine or stool. The specification does not contain working examples directed to the diagnosis of a tumor or risk of developing a metastasis in a single human test subject.

Predictability and state of the art: The art teaches that gene expression analysis is commonly used for three different purposes: (1) as a screening tool to identify individual genes of interest that might contribute to an important biological function, (2) to obtain insight into an important biological function, and (3) as a classification tool to sort cases into clinically important categories (Pusztai and Hess, Annals of Oncology, Vol. 15, pages 1731-1737, 2004, cited in a prior action; e.g., paragraph bridging pages 1732-1733). Pusztai and Hess teach that validation of gene expression important to biological function may be validated by using different methods, such as RT-PCR, whereas the most appropriate validation for using gene expression analysis as a classification tool is testing the predictor on independent sets of cases (e.g., page 1733, left column, 1st full paragraph). In the instant case the specification does not teach that the expression levels can be used to reliably categorize an individual (other than indicates in the allowable scope). For example, the specification does not teach the classification of individuals as at risk or not at risk to develop a metastasis, or at risk to develop a recurrence.

Further, Shalon et al (US 2001/0051344 A1, Dec 13, 2001, cited in a prior action) teach that due to variations in genetic make-up of unrelated individuals in a heterogeneous society, differences in the expression of a gene between any two individuals may or may not be significant (e.g., paragraph [0155]). Shalon et al further teach that the larger the number of individuals tested, the more significant the remaining differences in gene expression become and samples from at least 5 and preferably 20-50 different test individuals are assayed to obtain statistically meaningful data showing a statistical elevation or reduction in report levels when compared to control levels (e.g., paragraph [0156]). Pusztai and Hess teach that larger samples sizes may be needed to validate classification tests, and the number of samples will vary depending upon the acceptable error rates, level of inter-patient variability, the size of the difference in mean expression values, and the prevalence of the phenotype among the group being tested (e.g., page 1734, paragraph bridging columns; Table 1).

Genetic tests are heterogeneous in nature and the exact characteristics of a particular genetic test to be evaluated must be tightly defined (Kroese et al (Genetics in Medicine, Vol. 6, pages. 475-480, 2004, cited in a prior action). Kroese et al teach that genetic test is shorthand to describe a test to detect a particular genetic variant for a particular disease in a particular population and for a particular purpose and that it should not be assumed that once the characteristics of a genetic test are evaluated for one of these reasons that the evaluation will hold or be useful for other purposes and all measures of the test performance should be presented with their 95% confidence intervals (e.g., page 477, 1st column, 1st and 2nd full paragraph). Kroese et al teach that the limitations of our genetic knowledge and technical abilities means that

for the moment there are likely to be gaps in the information needed to complete a thorough evaluation of many genetic tests (e.g., page 479, 2nd column, last paragraph).

The prior art reveals that differences in gene expression observed between two groups are do not necessarily provide markers that can be used to reliably classify a subject. Golub et al (Science, Vol. 286, pages 531-537, October 1999, cited in a prior action) teach the use of a two-step procedure to test the validity of gene expression levels as predictors: step 1 involves cross-validation of the predictors on the initial data set, where one withholds a samples, builds a predictor based only on the remaining samples and predicts the class of the withheld sample; step 2 involves the repetition of assessing the clinical accuracy of the predictor set on an independent set of samples (e.g., page 532, right column). Although Golub et al could detect gene expression differences between chemotherapy responders and non-responders, those differences could not be use to predictably classify individuals (e.g., page 533, paragraph bridging left and middle columns). Accordingly, the art demonstrates the unpredictable nature of extrapolating gene expression differences to a method of class prediction.

The art teaches that different isoforms of MNSOD, TXNRD1 and GPX1 are expressed (See the Entrez Gene entries for SOD2, TXNRD1, and GPX1 downloaded from http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene on 5/5/2008, cited in a prior action). These genes correspond to the genes detected by the primers recited in the present specification. The specification does not specifically teach the increased expression of each isoform in circulating cancer cells. Thus, it would be unpredictable to detect any isoform of these genes for the use of the claimed method. Moreover, Seven et al (Clinical Biochemistry, Vol. 32, No. 5, pages 369-373, 1999, cited in a prior action) teach that the constitutive levels and the inducibility of

antioxidant enzymes including superoxide dismutase and glutathione peroxidase vary for different tissues, and the expression of these enzymes may vary according to the type of cancer or tissue studies, resulting in controversy in the literature (e.g., page 372, left column, last two paragraphs). Seven et al did not find an increased amount of CuZn SOD or glutathione peroxidase in the red blood cell fraction of laryngeal cancer patients (e.g., page 372, paragraph bridging columns; Table 1). Furthermore, the post filing art teaches that the expression of MNSOD, TXNRD1 and GPX1 measured by medium density micorarray and real-time RT-PCR showed a poor correlation (Giesing et al. BJU International, DOI: 10.1111/j.1464-410X.2009.08920.x , published online 10/10/2009, as pages 1-11; e.g., page 2, middle column). The microarray did not generate the necessary sensitivity and specificity to detect circulating cancer cells (e.g., page 3, right column, last full paragraph).

Amount of experimentation necessary: The quantity of experimentation necessary to carry out the full scope of the invention is large. One would be required to conduct a large number of experiments to test the expression of many manganese superoxide dismutase genes, thioredoxin reductase 1 genes, and glutathione peroxidase 1 genes, in combinations of two, from cell-containing fractions of body fluid, where the body fluid is blood or bone marrow, in a number of different species of organisms. Given the variable expression of the enzymes based upon tumor or cell type and the expression of multiple different isoforms, it would be unpredictable to extrapolate the results of the present specification to the use of any manganese superoxide dismutase gene, any thioredoxin reductase 1 gene, and/or any glutathione peroxidase 1 gene, and any comparative tissue. As discussed in the present specification, the limit used to determine whether a gene is overexpressed must be experimentally determined for each

particular comparison. This comparison will be specific for the organism, body fluid, cells collected from the body fluid, gene whose expression is determined, isoform whose expression is determined, whether mRNA or protein expression is measured, the specific method used to measure the mRNA or protein (e.g., RT-PCR, microarray, or ELISA), whether enrichment is used, and the type of control sample. A large amount of unpredictable experimentation would be required to use the full scope of the claimed method to detect the presence of disseminated cancer cells, provide diagnosis of a tumor, estimate the risk to develop a metastasis, or estimate the risk to develop a recurrence.

In view of the breadth of the claims and the lack of guidance provided by the specification as well as the unpredictability of the art, the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention. Therefore, claims 26-30, 32, 34, 36-60 and 62-70 are not considered to be fully enabled by the instant specification.

Response to Arguments - 35 USC § 112

The rejection of claims 47-49 under 35 U.S.C. 112, second paragraph, has been withdrawn in view of Applicant's amendment to the claims in the reply filed 5/12/2010.

The rejection of claims 31, 33, 35 and 61 under 35 U.S.C. 112, first paragraph, is moot in view of Applicant's cancellation of the claims in the reply filed 5/12/2010.

With respect to the rejection of claims 26-30, 32, 34, 36-60 and 62-70 under 35 U.S.C. 112, first paragraph, Applicant's arguments filed 5/12/2010 have been fully considered but they are not persuasive.

Any subject versus human subject

The response notes that all independent claims are now directed to a method for investigating a body fluid for disseminated cancer cells in a human subject.

Accordingly, the portions of the rejection directed to the practice of the method with subjects other than humans have been withdrawn.

Bone marrow versus blood

Presently, claims 26, 27, 29, 30, 32, 34, 36-44, 46-48, 50-52, 54-60, 62-66 and 68 are directed to the analysis of blood or bone marrow. Claim 28 is directed to the analysis of blood. Claims 45, 49, 53, 67, 69 and 70 are directed to the analysis of mononuclear cells (MNCs) from blood.

The response explains how disseminated cancer cells circulate in the body of an individual, including blood and bone marrow. Further, the response provides a theory or possible reasoning for the observed overexpression of antioxidant genes observed in the working examples.

The Examiner is not questioning the presence of disseminated cancer cells in the body of patients bearing tumors. Furthermore, a reason or biological mechanism that results in the overexpression of the antioxidant genes is not required for one to practice the claimed invention. The invention is based upon the comparative levels of gene expression products in various cell fractions and controls. The determination of disseminated cancer cells is based upon statistical analysis of gene expression data.

The response asserts that although the specification does not provide a working example for the investigation of bone marrow there is not reason to believe that the skilled person would

not have been able to practice the invention on bone marrow. The response asserts that this statement is confirmed in Prof. Giesing's Declaration of record under item 5.4.

These arguments are not found persuasive. The specification teaches that it is particularly important to determine whether expression in the cells of the investigated sample is comparatively elevated (e.g., page 28, lines 1-10). The specification teaches that the comparison usually is with cells in which no cancer-associated modification is to be expected (non-cancer cells, normal cells) (e.g., page 28, lines 10-14). The specification suggests that if cancer cells in body fluids are being tested, then the comparison will be those normally occurring in this body fluid. For the case of blood, the normal cells are white blood cells which can be obtained for example by density gradient centrifugation (e.g., the buffy coat or the MNC fraction) or can be separated by more specific isolation methods (e.g., CD45-positive lymphocytes) (e.g., page 28, lines 14-22). The specification asserts that these samples can also be used as a comparison for body fluids other than blood (e.g., page 28, lines 22-25). At page 29, lines 33-39, the specification states, "The test principle according to the invention is therefore based on determining whether enrichment of cancer cells is associated with a measurable increase in MNSOD, TXNRD and GPX expression. The ratio of the expression measured in the test cell mixture to the expression measured in the comparison cell mixture is decisive." The specification goes on to state, "It will usually be expedient for validation of a particular test system to fix a particular quotient (limit) above which overexpression is present by definition." (See page 30, lines 1-5). Thus, the step of comparing appears to be critical to the claimed invention. Furthermore, the specification notes that the limit may depend on the cell mixtures used and, in particular, on the obtaining thereof (e.g., page 30, lines 7-8).

The specification provides evidence to doubt that one could extrapolate the methods disclosed in the working example to other methods of cell-sampling and fraction isolation with blood or any other body fluid. For example, the specification teaches that the chosen method of enrichment of cancer cells may lead to the enrichment of non-cancer cells which show enhanced expression of the measured gene (e.g., page 30, lines 19-31). Enrichment for non-cancer cells that have enhanced expression of the measured genes will result in false-positive test results. This was the case observable for GPX1 expression when the white blood cells were subjected to a size- and shape-dependent separation process (e.g., page 30, lines 25-31). Accordingly, the comparison of gene expression levels is critical to the claimed invention, and the selection of body fluid, the selection of particular fractions, and method of cell enrichment will impact the outcome in an unpredictable manner. Accordingly, one could not extrapolate the results obtained in the working examples to other body fluids, other enrichment methods, or other cell fraction comparisons.

Section 5.4 of the Declaration of Prof. Giesing is a summary of experiments involving the analysis of SOD2, TXNRD1 and GPX1 expression levels in fractions of blood, where cancer cells were enriched using a mesh with 20 μm pores. This section also includes the opinion of Prof. Giesing that "While said studies have been carried out on blood and with respect to prostate cancer, it can reasonably be expected that essentially the same method can be applied to bone marrow and other cancer types." Thus, this section of the declaration does not provide evidence that the claimed methods would be successful using any other enrichment methods, any other blood fractions, or any other body fluids. The working examples of the specification provide evidence that the method may be applied to other cancer types. However, as discussed above,

the specification indicates that it would be unpredictable to vary the method of enrichment. Accordingly, it would be unpredictable to vary the body fluid.

Expression of the genes will be variable based upon the cell type selected. Thus, higher expression in the sample from the test subject will not be a reliable indicator of the presence of disseminated cancer cells. The specification does teach that it is particularly important to determine whether expression in the cells of the investigated sample is comparatively elevated (e.g., page 28, lines 1-10). The step of comparing is critical to the outcome of the invention, and it must be determined experimentally whether any particular comparison will provide a reliable indicator of the presence of a tumor or the risk of metastasis or recurrence. The Examiner has set forth evidence of the unpredictability of the invention above and in prior actions. Given the breadth of the claims, and the unpredictability in extrapolating the results disclosed in the working example to the full scope of the claimed invention, it would require a large amount of unpredictable experimentation to practice the full scope of the claimed invention.

Protein versus mRNA and RT-PCR versus microarray

The response notes that suitable methods for measuring protein and mRNA are known in the art and some methods are described in the specification (e.g., page 12, line 35 to page 14, line 16; page 20, line 25 to page 25, line 13).

The Examiner agrees that it is within the skill of the art to measure protein and mRNA expression levels. However, the claimed invention is not merely directed to the measurement of protein or mRNA expression levels. The claims require the levels to be predictive of disseminated cancer cells in a body fluid.

The response asserts that because protein expression is usually directly related to mRNA expression and AOX proteins may act in a survival and defense mechanism, one would be able to practice the claimed invention by measurement of protein.

This argument is not found persuasive. The specification teaches that there is wide variety in the proteins expressed from manganese superoxide dismutase genes, thioredoxin reductase genes, and human glutathione peroxidase 1 genes (e.g., page 14, line 35 to page 20, line 23). Consistent with the teachings of the specification, the art teaches that different isoforms of the claimed are expressed (See the Entrez Gene entries for SOD2, TXNRD1, and GPX1 downloaded from http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene on 5/5/2008, cited in a prior action). The specification does not specifically teach the increased expression of each isoform in circulating cancer cells. Thus, it would be unpredictable to detect any isoform of these genes for the use of the claimed method. Moreover, Seven et al (Clinical Biochemistry, Vol. 32, No. 5, pages 369-373, 1999, cited in a prior action) teach that the constitutive levels and the inducibility of antioxidant enzymes including superoxide dismutase and glutathione peroxidase vary for different tissues, and the expression of these enzymes may vary according to the type of cancer or tissue studies, resulting in controversy in the literature (e.g., page 372, left column, last two paragraphs). Seven et al did not find an increased amount of CuZn SOD or glutathione peroxidase in the red blood cell fraction of laryngeal cancer patients (e.g., page 372, paragraph bridging columns; Table 1). Furthermore, the post filing art teaches that the expression of MNSOD, TXNRD1 and GPX1 measured by medium density micorarray and real-time RT-PCR showed a poor correlation (Giesing et al. BJU International, DOI: 10.1111/j.1464-410X.2009.08920.x , published online 10/10/2009, as pages 1-11, cited in a prior action; e.g.,

page 2, middle column). The microarray did not generate the necessary sensitivity and specificity to detect circulating cancer cells (e.g., page 3, right column, last full paragraph). Accordingly, even different methods of measuring mRNA levels performed by the same group of scientists provide variable and unpredictable results.

The response asserts that the Giesing publication does not disqualify microarray technology as a suitable means for determining the expression of the genes at stake. The response asserts that it was only with the testing of 67 candidate RNAs that the results obtained with medium density micoarrays and quantitative real-time RT-PCR were found to show a poor correlation. Thus, the publication gave preference to RT-PCR.

This argument is not found persuasive. The 67 candidate RNAs included GPX1, SOD2 and TXNRD1 mRNAs now claimed (e.g., page 1, paragraph bridging left and middle column). Giesing et al state, "The AOX-test (SOD2, TXNRD1, GPX1) in CCC was selected out of 67 candidate genes using microarray technology (Genestick®). Real-time RT-PCR was chosen because the data did not correspond with Genestick®-microarray data (overlapping < 30%) thus generating the necessary sensitivity and specificity (data not shown)." See page 3, right column, last full paragraph. Accordingly, the Examiner has provided evidence that it would have been unpredictable to use micorarray analysis to measure expression of the claimed genes. Variation between the specific RT-PCR results and microarray analysis may be explained, in part, by the variety of isoforms expressed from the genes, which may be detected by microarray analysis. Applicant has not provided evidence to support the use of microarray analysis in the claimed invention.

Any MNSOD/TXNRD1/GPX1 gene versus the use of primers having SEQ ID NO: 1, 2, 4, 5, 7 or 8

The response asserts that the specification does not define manganese superoxide dismutase or MNSOD to mean enzymes belonging to the enzyme class 1.15.1.1, because this goes against the accepted meaning and the specification indicates that there are different superoxide dismutases.

These arguments are not found persuasive. Applicant has acted as his own lexicographer to specifically define the term "manganese superoxide dismutase (MNSOD)" to encompass both MNSOD and CuZnSOD even though this would be contrary to the ordinary meaning of manganese superoxide dismutase (MNSOD). The written description clearly redefines the claim term and sets forth the uncommon definition so as to put one reasonably skilled in the art on notice that the applicant has intended to so define the claim term "manganese superoxide dismutase." The definition is provided at page 14, lines 35-38. Evidence to support the enablement of MNSOD genes other than SOD2 amplified by the primers of SEQ ID NOs: 1 and 2 has not been provided.

Applicant notes that the claims are not directed to the measurement of MNSOD, TXNRD1 and GPX1 genes in humans. Further, the response asserts that the Declaration of Prof. Giesing and the results in Giesing et al. 2009 clearly show that the method of the present invention is enabled for any isoform that occurs in humans.

These arguments are not found persuasive. Multiple isoforms of the claimed genes may be found in humans, and the Declaration, Giesing et al publication, and specification all use the same primers for RT-PCR. These primers necessarily amplify the same isoforms. No evidence

has been provided to show that other isoforms are predictive in the claimed methods. Furthermore, a methods, such as microarray analysis, which is capable of measuring the expression of a variety of isoforms was found to not to generate the necessary sensitivity and specificity (Giesing et al. 2009, e.g., page 3, right column, last full paragraph).

Any method of cancer cell isolation versus using a screen with a mesh or pore width of about 20 μm

The response notes that the specification teaches that methods for isolating cancer cells were well-known in the art (e.g., specification, page 7, lines 15-21). Thus, the response asserts that a skilled person would have known how to isolate cancer cells without undue experimentation.

This argument is not found persuasive. The Examiner agrees that it would have been within the skill of the art for one to isolate cancer cells. The claimed methods are not merely drawn to the isolation of cancer cells. They require gene expression data from the obtained cell fractions to be predictive of disseminated cancer cells. The specification teaches that other methods of enrichment of cancer cells, such as size- and shape-selection, can result in the enrichment of non-cancer cells that also express the antioxidant genes (e.g., page 30, lines 19-31). This was especially found in relation to GPX1 expression when the white blood cells (mononuclear cells from blood) were subjected to a size- and shape-dependent separation process (e.g., page 30, lines 28-31).

Any test sample A' versus test sample A' from patients' own MNCs

The response essentially asserts that the claims are enabled for any further cell containing fraction if cancer cells are present in the body fluid under investigation.

This argument is not found persuasive. As discussed above, the expression of the claimed genes varies across different cell types and the method of selecting the cell fractions impacts the levels obtained. The type of comparison made is critical to the success of the claimed invention and variation in these parameters will lead to unpredictable outcomes.

Any elevated gene expression versus gene expression higher than a defined limit

The response asserts that the Examiner is of the position that the claimed method is enabled only if a limit for the gene expression (which is the average plus one standard deviation) is determined on healthy subjects. The response asserts that such a limit is not required for the practical application of the method; because once a test has been set up it is expedient to run the system on healthy individuals in order to determine the average expression of the genes at stake in cells from the body fluid of said healthy individuals. The response asserts that using such a limit is an advantage (as it helps avoiding false-positive results), but not a requirement for the method to be enabled. The response asserts that a skilled person would have readily recognized that once said average expression in healthy subjects has been determined for a given test system the claimed method can be carried out without the need for repeating said determination. It would make no sense if each time the method is used for investigating a body fluid from a patient having or suspected of having cancer it must also be carried out on a healthy subject. The response asserts that the current amendment reflects that a comparison with a pre-determined average gene expression (ratio) in subjects not having cancer is expedient and sufficient to enable the skilled person to determine the presence of disseminated cancer cells in the body fluid (i.e. in case of a significantly higher ratio of expression in the cell-containing fraction to the expression in the further cell-containing fraction as compared to the average expression in

subjects not having cancer (claims 47 and 48); or in case of a significantly higher expression in the cell-containing fraction as compared to the average expression in subjects not having cancer (claims 50 and 66)).

These arguments are not found persuasive. One would be enabled to practice the claimed methods using the exact same conditions disclosed in the working examples (including the use of the same cell line to determine cell equivalents of expression), where the limit for MNSOD is 1.2, TXNRD1 is 1.3, and GPX1 is 5.2. However, each of the claims is broader in scope than the method disclosed in the working example. For other conditions, the limits will need to be experimentally determined and cannot be predicted.

Tumor diagnosis and risk estimation for metastasis versus detection of disseminated cancer cells

With respect to tumor diagnosis and risk for metastasis, Applicant's arguments have been persuasive. The mere presence of circulating cancer cells would serve to indicate the presence of a tumor and potential risk of metastasis.

The state of the art and any unpredictability

The response asserts that the method of the present invention has been tested on independent states of cases (e.g., Giesing et al. 2009 and Prof. Giesing's Declaration of record).

This argument is not found persuasive. The Examiner has put forth a scope of enablement rejection. The data presented in the specification, Giesing et al. 2009 and Prof. Giesing's Declaration of record enables only a portion of the scope of the invention now claimed. The presented evidence is not commensurate in scope with the claims (e.g., regarding the

genes/isoforms measured, measurement of RNA/protein, particular enrichment of cancer cells, particular comparisons made, etc.).

The response asserts that the teachings of Kroese et al are not applicable, because the claims are not directed to directing heritable disease-related genotypes, mutations, phenotypes, or karyotypes for clinical purposes.

This argument is not found persuasive. Kroese et al teach test performance should be presented with their 95% confidence intervals (e.g., page 477, 1st column, 1st and 2nd full paragraph). Clinical utility of the test disclosed in the working examples of the present specification is described in terms of sensitivity and specificity (e.g., pages 46-47; page 57, lines 18-22). Giesing et al. 2009 also describe the AOX test as having a sensitivity of 86% and a specificity of 82%, with a positive predictive value of 69% and a negative predictive value of 92% accuracy (e.g., page 1, paragraph bridging middle and right column). Thus, the concepts set forth by Kroese et al are applicable to the presently claimed invention.

The response asserts that Golub et al does not demonstrate the unpredictable nature of the invention, but actually demonstrates the feasibility of cancer classification based solely on gene expression monitoring. The response recognizes that not all gene expression differences will be useful for class prediction and therefore Golub suggests the use of a two-step procedure to test the validity of gene expression levels as predictors.

The Examiner agrees with Applicant. Not all gene expression differences will be useful for class prediction. This is why the reference was cited. Gene expression differences do not equate to methods of classification. Tests must be validated for performance (e.g., positive predictive value, negative predictive value, etc). Such validation has only been performed for

the method disclosed in the present working examples, and these results cannot be extrapolated to the full breadth of the claims for the reasons set forth above and the reasons of record.

Applicant asserts that the teachings of Seven et al are not applicable to the claimed invention, because disseminated cancer cells are independent of the tumor entity from which they are derived.

Regardless of this possibility, Seven et al teach that constitutive levels and the inducibility of the antioxidant enzymes SOD, GSH Px, and catalase vary for different tissues (e.g., page 372, left column, 4th full paragraph). Thus, ratios obtained by the claimed methods will vary depending upon the selection of the comparative cell type, thereby making the outcome of the claimed methods unpredictable if the cell selection steps disclosed in the working examples of the specification are not used.

Applicant's arguments and the evidence of record have been fully considered but are not deemed persuasive in view of the record as a whole. Therefore, the claims stand rejected under 35 U.S.C. 112, first paragraph.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO

MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is (571)272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached on 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Jennifer Dunston/
Primary Examiner
Art Unit 1636